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Microalgae biochar-derived carbon dots and their application in heavy metal sensing in aqueous systems

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ABSTRACT

This research seeks a coupled solution for managing the large amounts of biochar produced by microalgae biofuels production, and the necessity for novel, economic and accurate heavy metal sensing methods. Therefore, this study evaluated the transformation of microalgae biochar (MAB) into carbon dots (Cdots) and their subsequent application as heavy metal ion sensors in aqueous systems. The experimental phase included the transformation of MAB into microalgae biochar-derived carbon dots (MAB-Cdots), MAB-Cdot characterisation and the evaluation of the MAB-Cdots as transducers for the detection of four heavy metal ions

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(Pb²⁺, Cu²⁺, Cd²⁺, and Ni²⁺). MAB–Cdot fluorescence was stable over a wide range of pH and resistant to photo-bleaching, making them suitable as fluorescence probes. The MAB–Cdot fluorescence was quenched by all of the metal ions and displayed different quenching levels. Depending upon the ions involved, MAB–Cdots were used to detect the presence of heavy metal ions from concentrations of 0.012 μM up to 2mM by measuring the reduction in fluorescence intensity. Neutral and slightly alkaline pHs were optimal for Cu²⁺ Ni²⁺ and Pb²⁺ heavy metal quenching. To quantify the concentration of the heavy metal ions, linear and logarithmic functions were used to model the MAB–Cdots fluorescence quenching. The sensing mechanism was determined to be reversible and purely collisional with some fluorophores less accessible than the others. This work demonstrated the ability to produce Cdots from microalgae biochar, examined their application as a transducer for detecting heavy metal ions in aqueous systems and paves the way for novel sensing systems using MAB-Cdots.

Keywords: Biochar; Carbon dots; Microalgae; Heavy metal ion; Fluorescence sensors; Quenching; Renewable nanomaterials

1 INTRODUCTION

During the last decade, interest in using microalgae as a feedstock for biofuel production has increased. The significance of microalgae biofuel production is based on microalgae's productivity (microalgae produce 2 to 10-times more biomass than that produced by terrestrial crops), ability to capture inorganic carbon as CO₂, use of marginal land for their cultivation, and significant lipid accumulation (4-60%) (Nam et al., 2016; Unkefer et al.,

2017). The production of biofuels from microalgae thermal conversion produced bio-oil products with improved heating values and lower oxygen/carbon ratios (Maguyon and Capareda, 2013; Maguyon-Detras and Capareda, 2017; Nam et al., 2017). Although microalgae upgraded bio-oil products have similar properties to some liquid fuels, bio-oil production corresponds to only 17 to 43% of the initial biomass. The remaining biomass is transformed into syngas (8 to 25%) and biochar (34 to 63%) (Maguyon and Capareda, 2013). Assuming a microalgae pond of 20.000 m² and a microalgae production of 600 kg of microalgae per day (Richardson et al., 2010), the thermal conversion of this biomass will produce between 79 and 140 tons of waste, or microalgae biochar, per year. The significant amount of biochar produced during this process is one of the hurdles in the industrialisation of the microalgae thermal conversion technology. To unlock the economic potential of this process, it is crucial to develop novel products and processes that can create a diverse portfolio of value-added products from the massive amounts of biochar.

As the necessity for developing biochar products has become imperative, novel processes have been reported for the transformation of biochar into different add-value products such as nanosilica, humic substances and carbonaceous nanomaterials (Genuino et al., 2017; Plácido et al., 2016; Plácido and Capareda, 2015). A promising class of nanomaterials is optically active carbon dots (Cdots). Cdots are zero-dimensional carbon materials with advantageous physical characteristics for applications such as optoelectronic and photonics (Ryu et al., 2013). Cdots have high solubility, outstanding photostability, favourable biocompatibility and low toxicity. In recent years, these materials have been fundamental in developing novel sensing methods for detecting different types of pollutants such heavy metal ions (Gao et al., 2017; Nair et al., 2017; Warriar and Kharkar, 2018).

67 The detection of heavy metal ions in aqueous systems is critical as heavy metal ions pose
68 serious threats to the environment and human health. Anthropomorphic activities have
69 increased the risk of heavy metal contamination and the occurrence of environmental issues,
70 creating the need for on-site, quick and accurate sensing methods for heavy metal ions
71 (Gogoi et al., 2015). Therefore, novel sensing techniques need to be able to detect heavy
72 metal ions below and above the regulation limits (Cd^{2+} 0.005 mg/L, Cu^{2+} 1.3 mg/L, Pb^{2+}
73 0.015 mg/L, Ni^{2+} 0.1 mg/L). The principle for heavy metal detection using Cdots is
74 fluorescence quenching, where the metal ions interact with the Cdots fluorophores groups
75 and reduce their fluorescence. The quenching effect is dependent on the metal ion-Cdot
76 interaction, which is different for each type of Cdot and metal ion (Gogoi et al., 2015; Gu et
77 al., 2016; Liu et al., 2016b; Song et al., 2015; Tiong et al., 2015; Ye et al., 2017; Zhang et al.,
78 2015). For instance, chicken egg white Cdots were only quenched by Fe^{3+} ions (Zhang et al.,
79 2015) while DNA-derived Cdots were quenched by both Hg^{2+} (20 μM) and Ag^+ (20 μM) ions
80 (Song et al., 2015). The fluorescence quenching of Cdots in the latter case was linear between
81 0 and 0.5 μM , and 0 and between 10 μM for Hg^{2+} and Ag^+ , respectively. Similarly, Hg^{2+} ions
82 had the most significant quenching effect on orange peel Cdots (Gu et al., 2016), pigeon
83 feather Cdots, pigeon manure Cdots, pigeon egg white Cdots and pigeon egg yolk Cdots (Ye
84 et al., 2017). Pigeon feathers Cdots were quenched by Fe^{3+} and Ag^+ while pigeon manure
85 Cdots were quenched by Fe^{3+} and Cu^{2+} (Ye et al., 2017). Chocolate Cdots were quenched by
86 Pb^{2+} with a linear relationship between 0 and 2 μM (Liu et al., 2016b). Clearly, differences in
87 the Cdot production process and input material influence the chemical structure and sensing
88 capabilities of the diverse array of Cdots.

While Cdots have been intentionally synthesised from microalgae biomass, this is the first report of Cdots derived from microalgae biochar (MAB-Cdots) and evaluated in heavy metal ion detection. Cdots produced from microalgae biomass have been used as fluorescent bioimaging probes for *Arabidopsis thaliana* and human breast cancer MCF-7 cells (Guo et al., 2017; Zhang et al., 2017). However, these microalgae-derived Cdots have not been evaluated for sensing heavy metal ions in aqueous systems. Thus, the need to evaluate the diversity of MAB-Cdot response to heavy metal ion exposure across a range of heavy metal ions. The use of MAB-Cdots and their further application as a heavy metal sensor is a coupled solution for managing the large amounts of biochar produced by biofuels production, and the necessity for novel, economic and accurate heavy metal sensing methods. Therefore, the aim of this article was to evaluate microalgae biochar from biofuels production as a raw material for MAB-Cdots production and the assessment of the potential of this advanced nanomaterial as a transducer for the detection of 4 heavy metal ions (Pb^{2+} , Cu^{2+} , Cd^{2+} and Ni^{2+}) in aqueous systems. The article includes the production and characterisation of MAB-Cdots as well as their evaluation as heavy metal ions sensors via fluorescence quenching.

2 MATERIALS AND METHODS

2.1 Substrate

Microalgae biochar (MAB) was used as a substrate for the production of microalgae biochar-derived carbon dots (MAB-Cdots). Dr Sergio Capareda and his Bio-Energy Testing and Analysis Laboratory (BETA Lab) at Texas A&M University kindly donated the MAB. MAB was produced from ground and dried *Nannochloropsis oculata* in a pyrolysis process using a batch pressure reactor at 500 °C for 30 min (Series 4580 HP/HT, Parr Instrument Company,

Moline, IL). After collecting the MAB from the pressure reactor, it was crushed using a mortar and sieved using a 1 mm mesh.

2.2 Chemicals

All chemicals were analytical grade: Potassium permanganate (KMnO_4) (Alfa Aesar), Acetone (Acros Organics), Nickel sulphate (Ni^{2+}) (Fisher Scientific), Copper sulphate (Cu^{2+}), Cadmium sulphate (Cd^{2+}), Lead Nitrate (Pb^{2+}), sodium dihydrogen phosphate, disodium hydrogen phosphate and Ethylenediaminetetraacetic Acid Tetrasodium (EDTA) salt (Sigma-Aldrich). Deionised and filtered (Milli-Q ultrapure water system with a $0.22\ \mu\text{m}$ filter, Merck Millipore) water was used in all the procedures.

2.3 Preparation of microalgae biochar–derived carbon dots

The biochar depolymerisation reaction was as follows: 10% solutions of KMnO_4 were mixed with microalgae biochar (5%) in 125 mL Erlenmeyer flasks. The depolymerisation was performed at $120\ ^\circ\text{C}$ for 1 hour at 15 psi in an autoclave (Med 12, Selecta) (Plácido and Capareda, 2015). After the chemical depolymerisation, the biochar solutions were centrifuged at 5000 rpm for 20 min (Legend RT, Sorvall) at room temperature to separate the liquid and solid phases. Acetone was mixed with the liquid phase until the production of a second liquid phase (Han et al., 2017; Varisco et al., 2017). The phases were separated by centrifugation at 5000 rpm for 20 min (Legend RT, Sorvall). The upper phase was withdrawn and roto-evaporated (miVAc Quattro concentrator, Genevac) until dry. After weighing, the solids were resuspended in ultrapure water and ultrasonicated for 1 minute at 50% amplitude (200W) (Branson, Emerson). The MAB–Cdots were obtained from the resuspended solids after

repeating the organic solvent precipitation process twice. The extracted MAB–Cdots were suspended in water and kept at 4 °C until use.

2.4 Characterisation of microalgae biochar-derived carbon dots

The MAB–Cdots were characterised with different spectroscopic and microscopic techniques. The fluorescence emission and excitation spectra of the MAB–Cdots were obtained on a Hitachi F2500 spectrophotometer. The stability of the MAB–Cdots fluorescence was measured at different pHs (pH 3 to 10) and exposure times (0 to 1h). FT–IR spectra were collected using a Frontier FT-IR spectrophotometer with sampler (PerkinElmer) from 4000–600 cm⁻¹ and the spectra analyses were performed using the Spectragryph software version 1.1 (Spectroscopy Ninja). UV-Vis absorption spectra were recorded using a U3310 spectrophotometer (Hitachi). Atomic force microscopy (AFM) images were captured on the BioScope AFM (BrukerCorporation.) in ScanAssistant mode and the image analysis was performed using the Bruker NanoScope software package v8.15 (Bruker Corporation), the particles were diluted to 100 ppm, filtered through a 0.2 µM filter and placed on mica substrate for AFM imaging. The MAB–Cdot size, zeta potential, and conductivity in solution were obtained by dynamic light-scattering (DLS) and laser doppler velocimetry (LDV) with the Zetasizer Nano ZS (Malvern). The measurements were performed using 0.2 µm filtered solutions in a DTS1070 cell with water as a dispersant (Refractive Index: 1.330) and assuming a MAB–Cdot refractive index of 2.418 (Nguyen et al., 2016).

2.5 Heavy metal ions quenching assays

Stock solutions of the metal ions were prepared at concentrations of at least 25 mM and for MAB–Cdots at concentrations of 1000 ppm. All the solutions were prepared using deionised and 0.22 µM filtered water. The metal titration quenching studies utilised MAB–Cdot solutions of 50 ppm diluted from the 1000 ppm solutions. The metal solutions were added to the cuvette containing MAB–Cdots starting from 0.0125 µM up to 20 mM. The range utilised was selected to include the minimum limit for these metals and concentrations reported on wastewaters effluents. Fluorescence spectra were collected after each heavy metal aliquot was added. The reduction in fluorescence was calculated as fluorescence reduction percentage (%) (see Equation 1).

$$\text{Fluorescence reduction \%} = \left(\frac{FL_0 - FL_{HMT}}{FL_0} \right) \times 100 \quad \text{Equation 1}$$

Where FL_0 is the MAB–Cdot fluorescence without the addition of heavy metal ions and FL_{HMT} corresponds to the MAB–Cdot fluorescence after a specific concentration of heavy metal was added. In the presence of Pb^{2+} , concentrations above 2 mM produced significant scattering and prevented accurate measurement. For Cu^{2+} and Ni^{2+} , it was possible to reach a concentration of 20 mM without generating significant scattering. To evaluate the pH effect on MAB–Cdot heavy metal quenching, MAB–Cdots were diluted in buffers of citrate or phosphate (5 mM) at pH 3, 4, 5, 6, 7, and 8 to reach a MAB–Cdot concentration of 50 ppm. Each metal solution was added to the cuvette containing MAB–Cdots to reach metal concentration of 50 µM. The fluorescence was collected before and after the addition of the heavy metal solution. The effect of buffer strength on MAB–Cdot/ heavy metal ions quenching was evaluated using 5 different concentrations of buffer phosphate pH 7 (2.5, 5, 10, 25, 50, 100 mM), MAB–Cdots were diluted in the buffers at 50 ppm. Each metal solution was added to the cuvette containing MAB–Cdots to reach metal concentration of 50 µM. The fluorescence reduction was calculated using **Equation 1**.

2.6 Fluorescence quenching mechanism

To understand the quenching mechanisms Stern–Volmer plots were constructed plotting F_0/F versus metal ions concentrations. Stock solutions of metal ions were prepared at concentrations of 100 mM, MAB–Cdots at concentrations of 1000 ppm and EDTA at concentrations of 100 mM. The metal quenching mechanisms studies utilized MAB–Cdots solutions of 50 ppm diluted from the 1000 ppm solutions. The fluorescence of the MAB–Cdots solution was measured and then the metal solutions were added to the cuvette containing MAB–Cdots (50 ppm) to reach a concentration of 50 μ M. The fluorescence of metal/MAB–Cdots solution was measured, followed by addition of EDTA to a final concentration of 100 μ M and after vigorous mixing fluorescence was measured. The fluorescence recovery was calculated using equation 2.

$$\text{Fluorescence recovery} = \frac{F}{F_0} \quad \text{Equation 2}$$

Where F is the fluorescence after the addition of the heavy metal or the EDTA and F_0 is the initial fluorescence of the MAB–Cdots.

3 RESULTS

3.1 Characterisation of microalgae biochar-derived carbon dots

The AFM microscopy describes the MAB–Cdots morphology (**supplementary material, Figure 1a**). The particle height was normally distributed with an average height of 4.7 ± 0.9 nm and the height ranged between 2.9 and 7.3 nm. The MAB–Cdots had a lateral dimension of 68 ± 25 nm with a range between 38 and 153 nm. The MAB–Cdots had an average hydrodynamic diameter of 175.5 nm, this diameter is close to the maximum lateral dimension

of the AFM. The MAB–Cdots had a zeta potential of -39.9 mV, which describes negatively charged molecules with moderate stability. A negative zeta potential facilitates interactions with positively charged particles such as heavy metal ions. MAB–Cdot FTIR spectra (**supplementary material, Figure 1c**) had several signals from different chemical linkages, the majority of these signals were associated with the presence of carbon linkages (1561, 1413, 1667, 2957, 2933, 2871, 719 and 648 cm^{-1} , **supplementary material, Figure 1c**). Bonds associated with aromatic carbons were the strongest signals (1561, 1413 cm^{-1}) and C–O and/or C=O linkages, C–H linkages, and aromatic linkages were more than 50% of the peaks identified. Additionally, MAB–Cdot FTIR spectra exhibited signals associated with sulphur, nitrogen and silica linkages. The peaks associated with aromatic, carboxyl and hydroxyl linkages were reported in Cdots from lignocellulosic material (Wang et al., 2017) and have been associated with the interaction between other carbonaceous materials and pollutants such heavy metal ions.

3.2 Fluorescence stability of microalgae biochar-derived carbon dots

As the interaction between MAB-Cdots and heavy metal ions is measured through the MAB-Cdot fluorescence reduction, it is important to characterise the fluorescent behaviour under different conditions. To understand the stability of the MAB–Cdot fluorescence, MAB–Cdot emission and excitation spectra were measured while modifying the pH, excitation and emission wavelengths, and time of exposure (**Figure 1**). MAB–Cdot fluorescence was observed for excitation wavelengths from the UV and extending until no emission was observed beyond 600 nm excitation. While generally consistent in the middle of the excitation range with a single emission peak at approximately 398 nm, the shape of the spectrum was different at an excitation wavelength of 280 nm. At the long excitation

wavelengths, the shape remained generally constant but with a shifted centre/decreased intensity (**Figure 1a**). Under excitation between 280 and 350 nm, the normalised emission curves narrowed without significantly changing the maximum emission wavelength of approximately 398 nm. Whereas under excitation between 350 and 450 nm, the maximum emission wavelength shifted, but the width remained constant. As the difference between excitation and emission wavelengths was not constant between 280 and 350 nm, the Stokes shift varied from 218 nm (280/498) to 148 (350/498) while it remained constant at 50 nm from 350 to 450 nm (**Figure 1b**). As the maximum emission was achieved at an excitation wavelength of 330 nm this wavelength was used in the further experiments. The MAB–Cdot fluorescence was strong, stable and almost constant over a wide range of pH (**Figure 1c**). These results indicated that MAB–Cdots could be used at different pH without generating a significant change in their fluorescent properties. MAB–Cdot photo-stability was evaluated by exposing MAB–Cdots to a continuous UV-Light illumination for 1 h with fluorescence collection every 5 min. The MAB–Cdots fluorescence intensity decreased less than 8% after 1 h (**Figure 1d**). These results showed that the MAB–Cdots are photobleaching resistant over the period of normal sample measurement.

3.3 Heavy metal ions quenching assay

To evaluate the applicability of MAB–Cdots to fluorescence-based metal ion sensing, fluorescence quenching experiments were performed with Pb^{2+} , Cu^{2+} , Cd^{2+} and Ni^{2+} (**Figure 2**). The four metal ions quenched the fluorescence of the MAB–Cdots. However, the quenching level and the metal concentration necessary to obtain quenching varied from metal ion to metal ion. Pb^{2+} was able to quench the fluorescence of MAB–Cdots up to 30% using around 2 mM of Pb^{2+} . At Pb^{2+} concentrations greater than 2 mM, MAB–Cdots generated

significant scattering and prevented extension of the measurement range. Pb^{2+} concentration and fluorescence reduction were linearly correlated using a logarithmic linearisation from 0.012 μM and 5 μM (**Figure 2a**, embedded figure). Cu^{2+} achieved the greatest quenching, producing a fluorescence reduction of more than 60% at 20 mM (**Figure 2b**). The Cu^{2+} concentration and the MAB–Cdot fluorescence reduction were linearly correlated between 1 μM and 10 mM using a logarithmic linearisation (**Figure 2b**, embedded figure). Cd^{2+} produced the lowest fluorescence quenching with a maximum fluorescence reduction of less than 10% (**Figure 2c**). Here, the quenching varied from 0.0125 μM up to 50 μM above which the fluorescence reduction saturated at around 8%. The Cd^{2+} concentration and fluorescence reduction were linearly correlated using a logarithmic linearisation between 0.01 μM and 1 μM . After Cu^{2+} , the Ni^{2+} quenching was the second largest quenching, producing a fluorescence reduction of nearly 50% at 20 mM (**Figure 2d**). Similar to Cu^{2+} , the Ni^{2+} concentration and MAB–Cdot fluorescence reduction was linearly correlated using a logarithmic linearisation between 3 μM and 10 mM (**Figure 2d**, embedded figure). **Table 1** describes the fitting parameters obtained from the heavy metal ions quenching experiments. MAB–Cdots could detect the broadest concentration range for Cu^{2+} and Ni^{2+} . Pb^{2+} and Cd^{2+} had the lowest concentrations (0.012 μM) with a response to MAB–Cdots. As Cd^{2+} did not produce significant quenching, additional tests were not performed for this metal.

Buffer solutions from pH 3 to 8 were used to evaluate the effect of pH on the MAB–Cdot fluorescence quenching from 50 μM of metal ions (**Figure 3a**). In Cu^{2+} and Pb^{2+} , the use of buffer solutions with lower pH produced a decrease in the quenching. The metal most affected by buffering was Pb^{2+} , where the maximum quenching in a pH 6 buffer solution (4.35%) decreased by 66% when compared to the quenching obtained in ultrapure water (14%). With Cu^{2+} , the maximum quenching in buffer solution (15%) was approximately half

that obtained in ultrapure water (27%). At low acidic pH, the quenching effect produced by the metal ions decreased by 85% to 95% (Cu^{2+} and Ni^{2+} 85% and Pb^{2+} 93%) when compared with the ultrapure water experiment and decreased by 73% to 85% when compared with the maximum quenching obtained with a buffer solution. The highest quenching was obtained at neutral and slightly basic or acid pH. However, the optimum pH was different for each metal ion (Cu^{2+} 7, Ni^{2+} 8 and Pb^{2+} 6). This process can be explained by the interaction among MAB–Cdot chemical groups, heavy metal ions and the buffer solution. Ni^{2+} reached the same level of quenching at pH 8 as with the experiment using ultrapure water. In all metals, low acidic pH was associated with low fluorescence quenching and was also associated with carboxyl and hydroxyl groups.

As buffer solutions reduced the fluorescence quenching, the buffer capacity was evaluated to identify their effect on MAB–Cdot fluorescence quenching (**Figure 3b**). Similar to the pH assays, the initial MAB–Cdot fluorescence was not influenced by changes of buffer strength (2.5-100mM) (data was not included). MAB–Cdot fluorescence quenched by Pb^{2+} was highly reduced by the addition of all amounts of buffer. After the initial reduction in fluorescence from the addition of low concentrations of buffer phosphate, higher concentrations did not produce any further fluorescence reduction. Similarly, increased buffer strength generated a negative effect on the MAB–Cdot fluorescence quenching in Cu^{2+} and Ni^{2+} , the change from 2.5 mM to 100 mM produced a reduction in the quenching ability of Cu^{2+} and Ni^{2+} of 10 and 5%, respectively. When compared with the quenching performed with ultrapure water, the quenching ability of Cu^{2+} and Ni^{2+} at 100 mM reduced almost 64 and 60 %, respectively. Although in all metal ions, 100 mM generated the lowest fluorescence reduction.

302

303 **3.4 Quenching mechanism**

304 The Stern-Volmer plots for the MAB–Cdot fluorescence quenching by heavy metal ions were
305 used to evaluate the fluorescence quenching mechanisms (**Figure 4**). In all cases, the
306 relationship between fluorescence quenching and the metal ions concentration were nonlinear
307 with a downward curvature. Such non-linear Stern-Volmer curves are characteristic of pure
308 collisional quenching where some of the fluorophores/quenching sites are less accessible than
309 others (Ke et al., 2018; Warriar and Kharkar, 2018). Thus, the MAB–Cdot Stern-Volmer
310 plots indicate a system having accessible and inaccessible quenching sites. Therefore, some
311 of the excited quenching sites in the MAB–Cdot structure experienced contact with the metal
312 ion facilitating the transition to the ground state, whereas other inaccessible quenching sites
313 maintained their excited state as they could not interact with the heavy metal ions. The
314 inaccessible quenching sites can be hidden in the MAB–Cdot structure due to MAB–Cdot
315 interactions or by other constituents in the MAB–Cdots. The most efficient quencher was
316 Cu²⁺ followed by Ni²⁺ and Pb²⁺. The differences observed in the Stern-Volmer plots indicated
317 significant differences in the interactions between MAB–Cdots and the metal ions

318

319 To understand the strength of the interactions between MAB–Cdots and the metal ions,
320 EDTA was used to evaluate whether it is possible to recover the fluorescence after metal ion
321 addition (**Figure 5**). As shown in the embed image, it was possible to completely recover the
322 initial MAB–Cdot fluorescence in the system after using 100 µM of EDTA (2:1 EDTA:Metal
323 ions). A *t-test* was used to demonstrate the MAB-Cdots fluorescence recovery after EDTA
324 addition (**Supplementary material, Table 1**). The full fluorescence recovery demonstrated
325 that the metal ions had a weaker interaction with MAB-Cdots as compared to EDTA.

Therefore, a chelating agent with greater binding energies for the metal ions, such as EDTA, strips the metal ions from the MAB–Cdot quenching sites and enables the fluorophores/quenching sites to be re-used.

4 DISCUSSION

This is the first article reporting the production of Cdots from biochar originated from microalgae thermal conversion looking to couple advanced materials production with environmental sensing. As a potential application for this value-added product, the use of MAB-Cdots as a transducer for the detection of heavy metal ions was explored. Previously, Cdots have been produced from raw microalgae as a primary product and effectively used as a fluorescent bioimaging probe (Guo et al., 2017; Zhang et al., 2017). Compared with the methods previously reported for the primary production of microalgae Cdots, the method reported in this article not only provides a value-added product from industrial waste, but is also a simpler and faster approach using a two-step method with 4 h of processing time to produce MAB–Cdots. The optical properties of the MAB–Cdots, such as photobleaching resistance, pH stability and negative zeta potential, support their use as a detection probe for heavy metal ions (Gu et al., 2016; Liu et al., 2016b; Ye et al., 2017). Photobleaching is the alteration of a fluorophore that makes it permanently unable to fluoresce. Although the MAB-Cdots fluorescence reduced after one hour of continuous exposure to UV-Light (330 nm), the fluorescence decreased 10% of the original value. This makes MAC-Cdots more resistant to photobleaching than other organic fluorophores (e.g. FITC) which completely lose their fluorescence after seconds to minutes of continuous illumination exposure (Mahmoudi et al., 2011). Similar to MAB-Cdots, photobleaching resistance is a common property in other renewably produced Cdots used for heavy metal ion sensing such as Cdots derived from

feather wastes (Ye et al., 2017) and DNA derived fluorescent bio-dots (Song et al., 2015). The photobleaching observed in the MAB-Cdots did not influence individual quenching measurements because the MAB-Cdot exposure to UV-Vis light in this process was less than 30 seconds for each the initial and quenching measurement. In fact, this level of photobleaching resistance enables multiple measurements of this system over time without introducing measurement error.

The different quenching levels and dynamics registered by each heavy metal ion can be associated with chemical, electronic and vibrational properties (Shtepliuk et al., 2017). As evidenced by the FT-IR spectra, MAB-Cdot structure is rich in C–O, C=O and C–OH linkages, indicating similarities to graphene oxide dots and their interactions with heavy metal ions. Although work on other Cdots has pointed to the presence of amino groups as responsible for the interaction with heavy metal ions specially Cu^{2+} (Liu et al., 2016a), MAB-Cdots did not have a significant presence of amino groups in their structure (supplementary material). The MAB-Cdot/heavy metal ion interactions involve MAB-Cdots functional groups with unshared electron pairs capable of forming coordination linkages with the heavy metal ions. The most important groups are carboxylic acids and phenolic OH. Carboxylic and phenolic groups are hard donors with electrostatic forces and entropy gains driving their interactions with heavy metal ions. The high affinity between carboxylic acids and Pb^{2+} , Cu^{2+} , and Ni^{2+} ions is well known and has been proved in other material systems such as humic substances (Perelomov et al., 2018), (Zhou et al., 2018) hydrogels and acetic acid films (Xu et al., 2016).

Table 2 compares MAB-Cdots with different renewably produced Cdots used for detecting heavy metal ions in terms of the production method, the heavy metal ion detection, the

detection range and the limit of detection. The advantage of using residual biochar from the bioenergy industry introduces the possibility of coupling bioenergy and nanomaterials production from waste. In contrast, all the production methods for other renewable Cdots produce the Cdots as the primary product and require significant energy input into the process. Additionally, biochar-derived carbon dots can be produced from the bioenergy production from several other feedstocks beside microalgae. Pb^{2+} , Cu^{2+} and Ni^{2+} all significantly quenched MAB-Cdots. In contrast, Hg^{2+} and Pb^{2+} are the most common heavy metal ions with significant quenching in other renewably produced Cdots. The advantage of significant quenching by only one or two types of heavy metal ions is the reduction of selectivity issues. The complexity of selectivity in MAB-Cdots need to be addressed in the future. On the plus side, MAB-Cdots were quenched by both Ni^{2+} and Cu^{2+} . Ni^{2+} did not significantly quench other renewably produced Cdots, and Chitosan hydrogels-Cdots were the only known Cdots to be quenched by Cu^{2+} . The detection ranges of heavy metal ions were generally wider for the MAB-Cdots than the other renewable Cdots. As shown in **Table 2**, this wide range will allow their use in highly polluted environments (mine pollution or wastewaters effluents) (1 mg/L to 500 mg/L) and in low polluted waters for detecting heavy metal ions close to the permitted concentrations (Cd^{2+} 0.005 mg/L, Cu^{2+} 1.3 mg/L, Pb^{2+} 0.015 mg/L, Ni^{2+} 0.1 mg/L). The limits of detection for MAB-Cdots were similar to those reported by other renewably produced Cdots, below 0.1 μM . Similar to other renewably produced Cdots, MAB-Cdots were affected by pH and buffer strength. At an acidic pH, the MAB-Cdot hydroxyl ($-\text{OH}$) and carboxyl linkages ($-\text{COOH}$) shift from the charged form ($-\text{OH}^-$ and $-\text{COO}^-$) into an uncharged form ($-\text{OH}$ and $-\text{COOH}$). These changes in MAB-Cdot charge reduce the interaction points between the heavy metal ions and MAB-Cdots altering the quenching effect produced by the heavy metal ions.

399 The high affinity between heavy metal ions and MAB–Cdots also causes selectivity issues.
400 Therefore, it is necessary to improve the MAB–Cdot selectivity for developing a sensing tool
401 with an accurate determination and discrimination between heavy metal ions. Following
402 published work in the field, the MAB–Cdot selectivity can be improved through a number of
403 different approaches. First, the inclusion of phosphorous or nitrogen groups in the Cdot
404 structure improved the selectivity in pidgeon feather derived Cdots (Ye et al., 2017) and lotus
405 root derived Cdots (Gu et al., 2016). Second, coupling a secondary set of materials to
406 improve the selectivity has been shown in different types of Cdots or hydrogels (Gogoi et al.,
407 2015). Third, the inclusion of an additional set of measurements complementing the
408 fluorescence quenching and use multivariate statistics for heavy metal discrimination has
409 been an effective approach (Lippolis et al., 2018).

410

411 In all cases, the Stern-Volmer plots for the MAB–Cdot fluorescence quenching were
412 nonlinear with a downward curvature. This type of behaviour is different from that reported
413 by other renewably produced Cdots. Pidgeon feathers derived Cdots and orange peel derived
414 Cdots had linear behaviours that fit the traditional Stern-Volmer model ($F_0/F = K_{sv}[C] + 1$).
415 Orange peel derived Cdots were linear at concentration similar to those used in the MAB–
416 Cdots. However, the Stern-Volmer linearity in pidgeon feather derived Cdots was only valid
417 at low concentrations. At low concentrations (0 to 5 μM), the Stern-Volmer plot for the
418 MAB–Cdots/ Cu^{2+} quenching is linear, whereas Ni^{2+} and Pb^{2+} kept the non-linear downward
419 behaviour. In Stern-Volmer plots, linearity deviations may be associated with the quencher,
420 diffusion properties or the local concentration of the quencher around the quenching sites (Ke
421 et al., 2018; Warriar and Kharkar, 2018). Therefore, the affinity of the heavy metal ions for
422 carboxylic and phenolic OH groups are responsible for some of the MAB–Cdot fluorescence

quenching. The interaction sites not quenched can be associated to a lack of interaction with the metal ions by steric effects or chemical repulsion.

The environmental sensors market is estimated to be worth 1.37 billion dollars and the stringent environmental regulations will be pushing the development of more accurate and portable sensors and less impact of human activities on the environment. Therefore, there is a market for MAB-Cdot derived biosensors, produced from renewable. Future work will focus on modelling the quenching process, improving the selectivity and accuracy of MAB-Cdots and analysing the economics of integrating MAB-Cdots production with that of biofuels.

5 CONCLUSIONS

This article studied the use of microalgae biochar as a raw material to produce Microalgae Biochar-derived Cdots and the use of this nanomaterial as a transducer for the detection of heavy metals in aqueous systems. MAB-Cdots fluorescence was significantly quenched by Pb^{2+} , Cu^{2+} and Ni^{2+} . The correlation between fluorescence reduction and metal ion concentration was established for each metal ion including the limits of detection ($0.01\mu\text{M}$ Pb^{2+} , $0.1\mu\text{M}$ Cu^{2+} and $0.1\mu\text{M}$ Ni^{2+}) and range of detection ($0.01\mu\text{M} - 2\text{mM}$, Pb^{2+} ; $1.5\mu\text{M} - 10\text{mM}$, Cu^{2+} and $2.5\mu\text{M} - 10\text{mM}$, Ni^{2+}). The Stern-Volmer plots implied the presence of accessible and inaccessible interaction sites in the MAB-Cdot structure while EDTA studies suggested loose interactions and reversibility of the sensor system. This is the first step in the development of a novel and sustainable method for environmental sensors from microalgae biochar and the integration of nanomaterial production into microalgae biofuels production.

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FIGURES AND TABLES LIST

Table 1. Linearisation parameters for heavy metals quenching using MAB–Cdots

Table 2. Comparison between MAB-Cdots and previously reported renewably produced Cdots as heavy metal sensors.

Figure 1. a) MAB–Cdots emission spectra at different excitation wavelengths b) MAB–Cdots maximum fluorescence and max emission wavelength at different excitation wavelengths. b) c) MAB–Cdots fluorescence at different pHs d) MAB–Cdots fluorescence at different illumination times.

Figure 2. FL emission spectra of MAB–Cdots in the presence of different concentrations of heavy metals a) Pb^{2+} b) Cu^{2+} c) Cd^{2+} d) Ni^{2+} . Embedded images show the dependence of $(I_0 - I)/I_0$ on the concentrations of heavy metals

Figure 3. MAB–Cdots FL-quenched efficiency $(I - I_0)/I_0$ a) at different pHs b) at different phosphate buffer pH 7 concentrations

Figure 4. Stern-Volmer plots for MAB–Cdots quenching using 50 ppm of MAB–Cdots and different concentrations of metals (0.0125 μM to 20 mM) a) Cu^{2+} b) Ni^{2+} c) Pb^{2+}

Figure 5. MAB–Cdots fluorescence emission spectra FL in the absence and the MAB–Cdots fluorescence recovery, 50 μM of heavy metal and FL restoration in the presence of 100 μM of a) Cu^{2+} b) Ni^{2+} c) Pb^{2+} .